Supplemental Material for

Modeling the Signatures of Hydrides in Metalloenzymes:

ENDOR analysis of a di-iron Fe(μ-NH)(μ-H)Fe core

R. Adam Kinney,[†] Caroline T. Saouma,[‡] Jonas C. Peters,[*,‡] and Brian M. Hoffman[*,†]

[†]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, USA

[‡]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, USA

Variable Mixing-Time (VMT) Davies ENDOR.

For an $S = 1/2$, $I = 1/2$ system, as the mixing time is increased, the ENDOR response associated with the $m_s = -^1/_2$ electron spin manifold (β) decreases more rapidly than the response associated with the $m_s = +^1/_2$ manifold (α). By comparing the relative intensity of the ν_+ and ν_- ENDOR transitions for Davies ENDOR spectra acquired at different t_{mix}, we can match each transition to their corresponding electron spin manifold. Comparison of the ^{1}H Davies ENDOR spectrum with the VMT-ENDOR spectra (t_{mix} = 3, 8 ms) for the imido proton reveals that the intensity of ν_+ decreases at a faster rate than ν_- (**Fig. S2**, *inset*), indicating that ν_+ is an α transition, and confirming the sign assignments made from the PESTRE measurements.

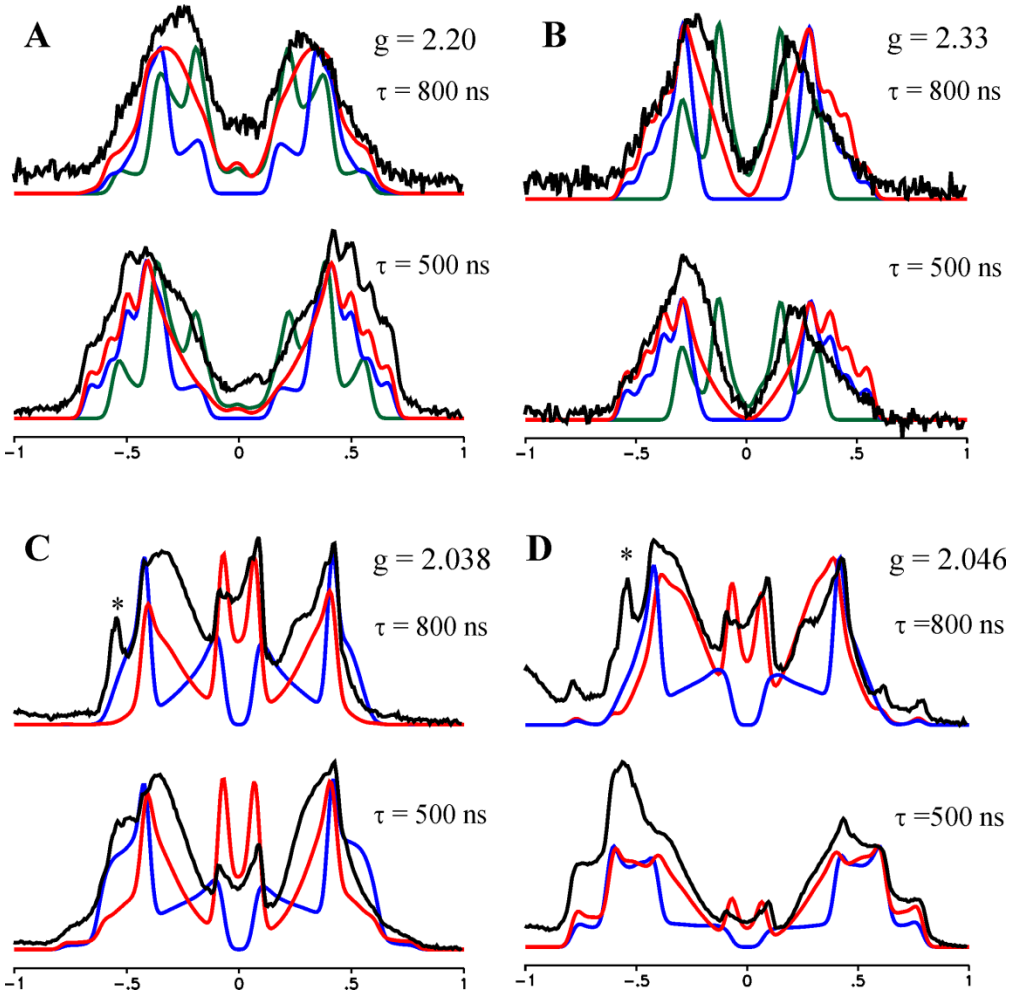

Figure S1. Pulsed ^{2}H Mims ENDOR of $^{2}H_{NH}$ at field positions corresponding to the regions g_1-g_2 (**A**, **B**), g_2 (**C**), and g_2-g_3 (**D**). Simulations are for different hyperfine tensors: [0.5, 1.4, -0.5] (red); [0.5, 1.4, 0.5] (blue); [-0.5, 1.4, 0.5] MHz (green). Experimental spectra are in black. *Experimental conditions*: microwave frequency, 34.734 GHz; repetition rate, 20 ms; tau values are as listed for each set of spectra. *Simulation parameters*: hyperfine and tau values are as listed above; (α, β, γ) = (100, 6, 90) (g_1 = z); EPR linewidth, 200 MHz; ENDOR linewidth, 0.05 MHz, RF randomly hopped.

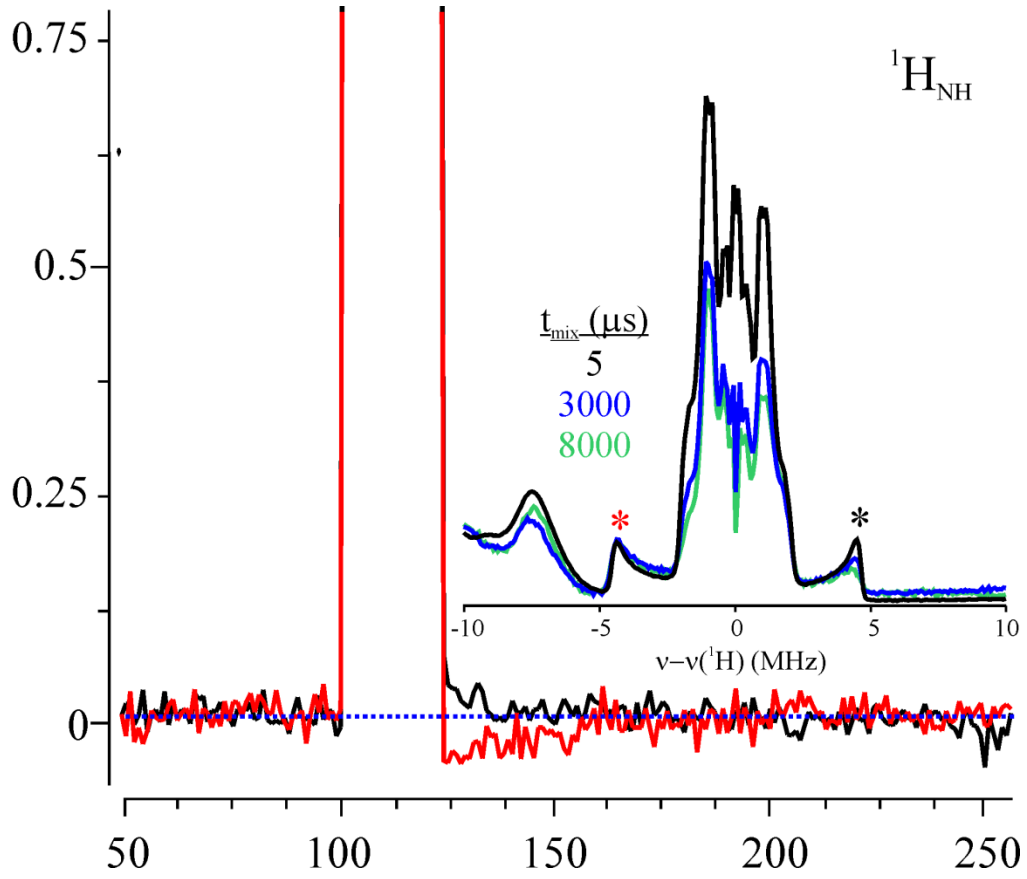

Figure S2. PESTRE trace acquired from **3** at the ν_+ (56.3 MHz; black) and ν_- (47.4 MHz; red) frequencies from the ^{1}H ENDOR response at $g_2 = 2.05$. *Inset*. VMT-Davies ^{1}H ENDOR spectrum at g_2. The frequencies at which a PESTRE spectrum was acquired are denoted by stars. *Experimental conditions*. PESTRE: microwave frequency, 34.892 GHz; π = 200 ns; τ = 600 ns; repetition rate, 20 ms; t_{rf} = 30 μs; t_{mix} = 5 μs. VMT-ENDOR: Microwave frequency, 34.898 GHz; π = 200 ns; τ = 600 ns; repetition rate, 20-28 ms; t_{RF} = 30 μs; RF frequency randomly hopped.

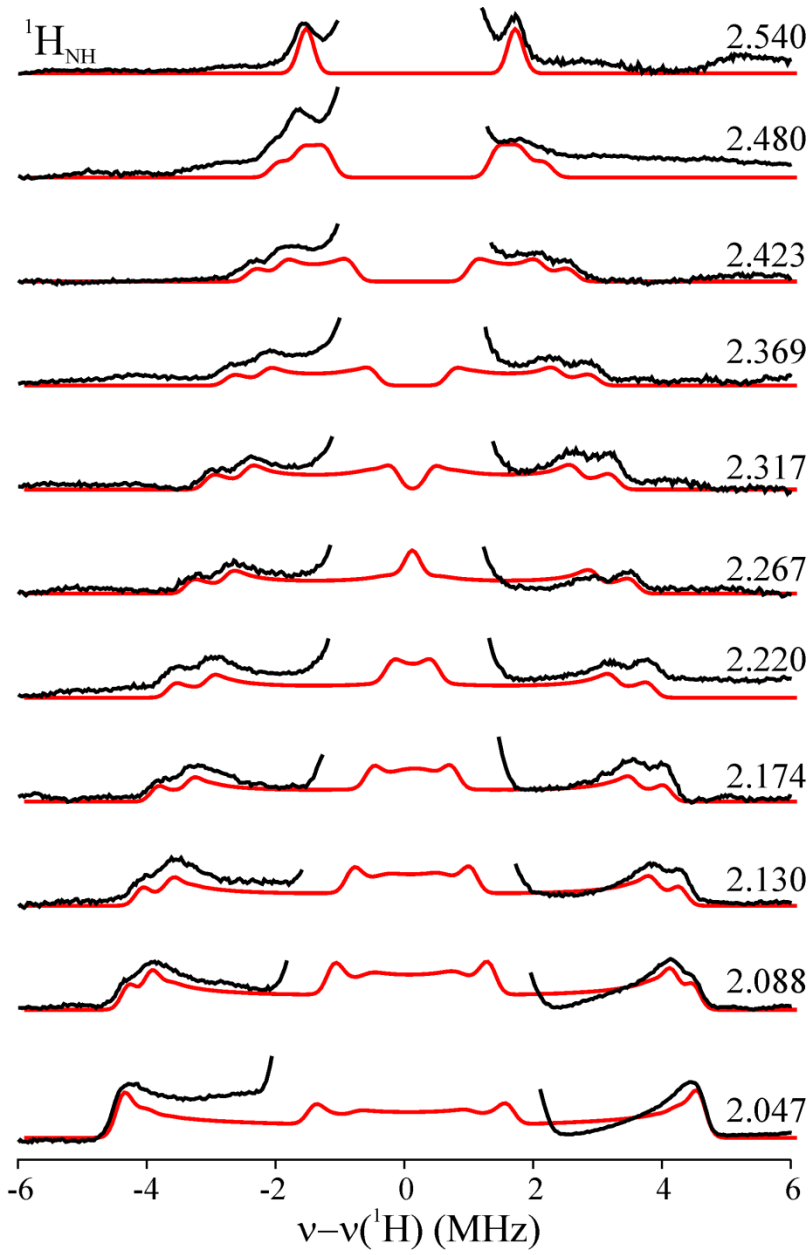

Figure S3. ^{1}H swept (low to high frequency) CW ENDOR field-frequency pattern for the imido ligand proton (H_{im}) of **3**(NH/D). Simulations (red) were generated directly from the ^{2}H simulations by scaling the hyperfine coupling principal values by the ratio of the ^{1}H and ^{2}H nuclear g values. *Experimental conditions*. Microwave frequency, 35.232 GHz; microwave power, 10 mW; modulation amplitude, 0.66 G; time constant, 8 ms; RF power, 10 W; temperature, 2K; and the bandwidth of RF excitation was broadened to 50 kHz. *Simulations*: **g** = [2.54, 2.047, 2.031]; **A** = -[3.2, 9.1, -3.3] MHz; (α, β, γ) = (90, 6, 100); EPR/ENDOR linewidth 200/0.2 MHz.

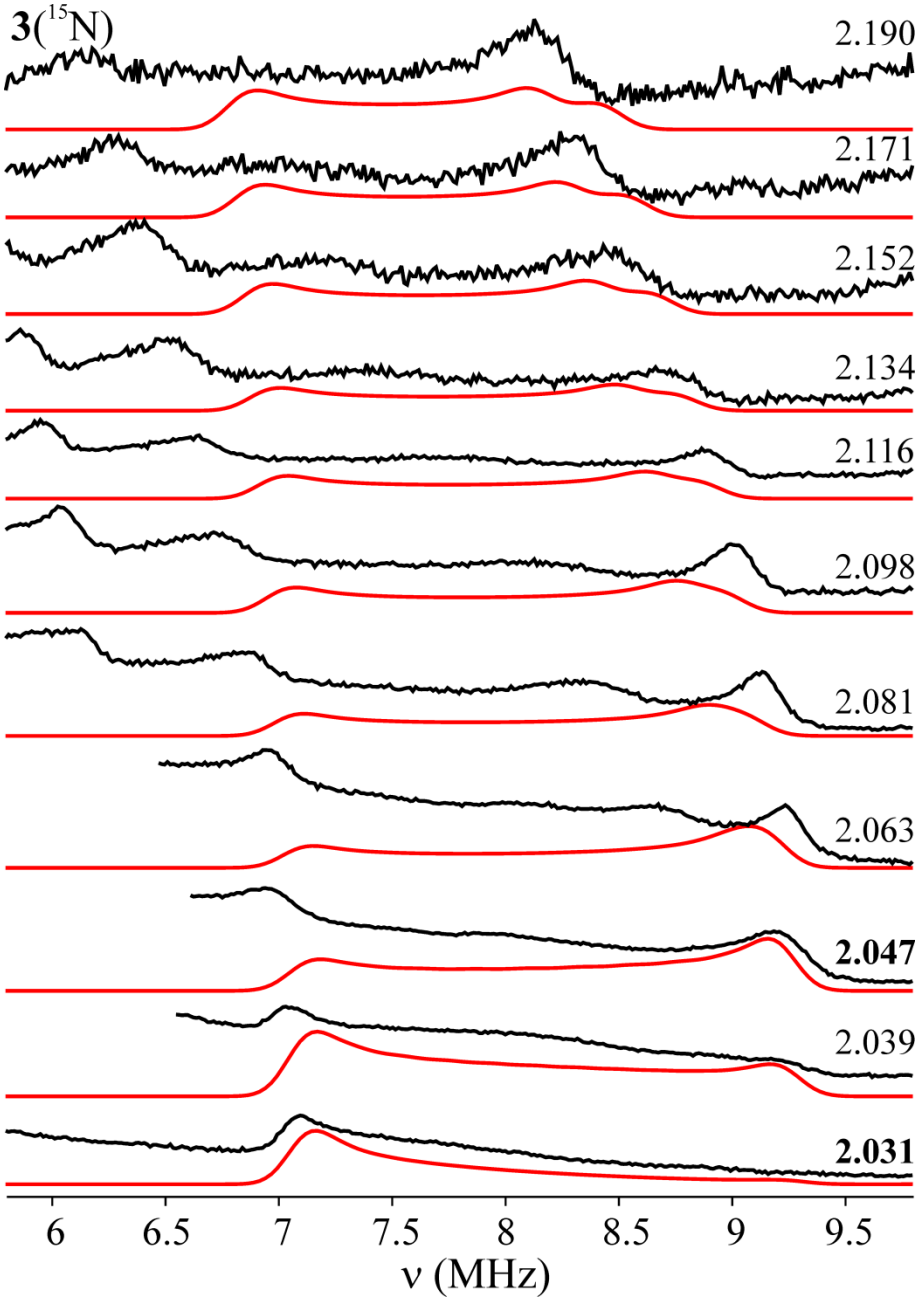

Figure S4. ^{15}N Davies field-frequency ENDOR pattern from **3**(^{15}N). *Experimental conditions*. Microwave frequency, 34.99 GHz; repetition time, 20 ms; t_{RF} = 30 μs; τ = 600 ns; RF randomly hopped. *Simulations*. **g** = [2.54, 2.047, 2.031]; **A** = +[4.1, 8, 3.5] MHz; (α, β, γ) = (90, 5, 90) (g_1 = z), EPR/ENDOR linewidth, 300/0.2 MHz, ν_+ manifold only.

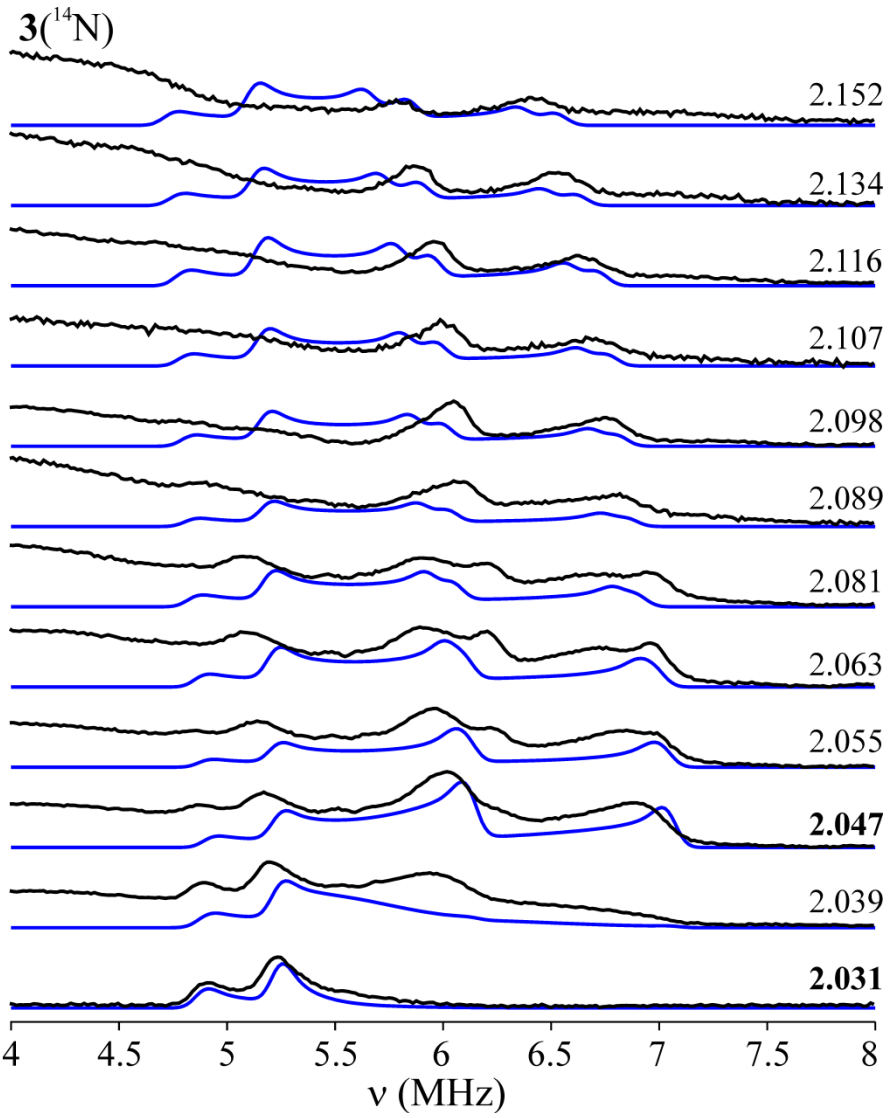

Figure S5. ^{14}N Davies field-frequency ENDOR pattern from **3**. *Experimental conditions*. Microwave frequency, 34.994 GHz; repetition time, 15 ms; t_{RF} = 15-30 μs; τ = 600 ns; RF randomly hopped. *Simulations*. **g** = [2.54, 2.047, 2.031]; **A** = -[3, 5.7, 2.5] MHz; (α, β, γ) = (90, 5, 90) (g_1 = z); **P** = [0.19, -0.31, 0.12] (coaxial); EPR/ENDOR linewidth, 150/0.1 MHz, ν_+ manifold only.